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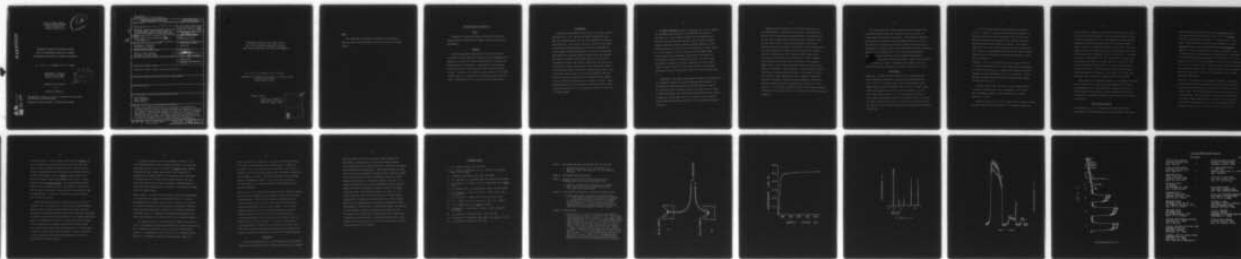
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PROCEDURAL INNOVATIONS AND MEMORY EFFECTS
WITH THE MICRO-SAMPLING NEBULIZER TECHNIQUE
FOR PREMIXED FLAME ANALYTICAL ATOMIC SPECTROMETRY

R. C. Fry, S. J. Northway, and M. B. Denton

Department of Chemistry
University of Arizona
Tucson, Arizona 85721

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R.C. Fry¹, S.J. Northway, and M.B. Denton*

Department of Chemistry and University Analytical Center
University of Arizona
Tucson, Arizona 85721

¹Present address:

Department of Chemistry
Kansas State University
Manhattan, Kansas 66506

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BRIEF

New procedures are described for employing micro-sampling nebulizer cones, and burner memory effects are shown to be greatly reduced.

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TITLE

Procedural Innovations and Memory Effects with the Micro-sampling Nebulizer Technique for Premixed Flame Analytical Atomic Spectrometry.

ABSTRACT

Studies are presented describing new "on-the-spot" micro-calibration (standard addition) and micro-dilution procedures and apparatus to be used with a small volume nebulizer technique for analytical atomic spectrometry. Improvements in burner memory problems are shown to result when the micro-sampling technique is used. Tolerance of the method to complex matrices is evaluated, and applicability is demonstrated to premixed flame emission and atomic fluorescence as well as atomic absorption spectrometry.

INTRODUCTION

Although the development of high sensitivity thermal (Delves' cup, boat, etc. (1,2)) and electro-thermal (graphite furnace, carbon rod, tantalum strip, etc. (3,4,5)) atomization, ultrasonic nebulizers (6,7), "high solids" nebulizers (8) and "on line" pre-concentration (hydride generation, etc. (9,10)) techniques may provide greatly improved performance in a variety of specialized applications, the combined premixed flame and capillary pneumatic nebulizer will probably continue for some time to be the most "purchased" and most "used" sample introduction technique for general analytical atomic spectroscopy of most samples and elements. The improved operational convenience, reduced analysis time, level of precision, and low cost of the capillary nebulizer generally make it the method of choice unless the samples are too complex, too low in analyte concentration, or too small in volume. These extreme conditions will then dictate the tradeoff of one or more desirable characteristics of the pneumatic nebulizer in favor of a different method.

The atomic absorption studies of Sebastiani et. al. (11), Manning (12), and Berndt and Jackwerth (13) demonstrate that samples in the range of 15 - 200 μ L may be pipetted into non-wetted plastic sampling cones mounted on the capillary tube of a premix capillary pneumatic nebulizer yielding transient recorder responses of precision and sensitivity similar to that achievable with normal larger (1 - 5 mL) samples. This is a result of the "zero dead volume" of such cones and the hydrophobic cone material that enable the entire sample to enter the nebulizer capillary in a single "plug". The method is reported to be rapid, convenient, and less susceptible to system clogging (11) than normal steady state aspiration techniques since less total amount of matrix is aspirated per analysis.

Regardless of the sample introduction technique used for analysis, it is generally desirable to analyze digested complex materials by the method of standard additions to avoid the deleterious results of calibration curve alteration caused by a variety of matrix effects. It should be apparent that standard volumetric glassware used to make standards or standard additions prior to analysis tend to require larger samples and defeat the purpose of a micro-sample introduction technique.

Semiquantitative estimation of the analyte concentration is also desired before a standard addition analysis can proceed on a sample of totally unknown analyte concentration. When coupled with the fact that a large number of samples of totally unknown content may vary over as much as four orders magnitude of analyte concentration when the instrument is adjusted for a given range covering only one or two orders of magnitude simultaneously, this presents a rather inconvenient logistics problem if large numbers of unknown small volume samples are to be analyzed. Either a separate set of analyses must be made requiring storage of micro-samples between times, or instrumental operation must be continually interrupted for "wet bench" standard addition and/or dilution operations following each semiquantitative estimate. The net result is that many "high work load" laboratories avoid the method of additions altogether or attempt to make up working standards in artificially concocted matrices designed to approximate the composition of the samples. An "on-the-spot" micro-addition and micro-dilution procedure is therefore clearly desirable from a logistics and sample conservation standpoint.

This paper deals with further evaluation of the characteristics of the micro-sampling nebulizer technique as well as several pertinent design and procedural innovations. A new method is presented for convenient ("on-the-spot") micro-standard additions and micro-dilutions to be performed by the analyst without interrupting instrument operation. Several common burner memory effects are evaluated and found to play a different role in the transient micro-sampling approach than would normally occur with steady state sampling.

The micro-sampling premix nebulizer technique is found to be applicable to flame emission and atomic fluorescence spectrometry as well as the atomic absorption applications originally reported (11, 12, 13).

EXPERIMENTAL

Apparatus. A Varian Techtron (Springvale, Vic., Australia) AA-5 atomic absorption spectrometer equipped with standard nebulizer, 10 cm and 6 cm slot burner heads, circular burner head, hollow cathode lamps, and Hewlett Packard (Palo Alto, Calif.) model 17501A strip chart recorder was used for these investigations. Cd, Zn, and Tl Osram lamps, Gates (L.I., N.Y.) power supply, and 25 mm focal length, 25mm diameter fused silica condensing lens are provided for atomic fluorescence excitation. Normal hollow cathode and flame excitation were used for atomic absorption and flame emission analyses respectively.

Two Teflon sampling cones were constructed as diagrammed in Fig. 1. Cone "A" is similar to that described elsewhere (12) except that connection to the instrument is via the small bore vertically oriented plastic nebulizer tubing rather than direct mounting on the horizontal nebulizer capillary. The tubing is "press-fit" into a hole drilled vertically through the apex of the cone. Cone "B" was developed in these studies for smaller sample sizes as well as "on-the-spot" micro-standard addition and micro-dilution procedures.

Procedure. Fuel rich air-acetylene ($2.2 \text{ L/min C}_2\text{H}_2$) flames and a nitrous oxide-acetylene ($\sim 6.6 \text{ L/min C}_2\text{H}_2$) flame were maintained at oxidant flows of 12 L/min . Samples were digested according to standard dry ashing procedures for Cu, Fe, and Zn (14). Silicon analysis was made following digestion of petrified wood samples in 30% hydrofluoric acid.

Samples are delivered into cone "A" using Eppendorf pipets. A transient recorder signal results immediately. Peak height was the parameter measured. The instrumental time constant (5RC) was $\sim 0.5 \text{ sec}$.

Samples pipetted into cone "B" may be diluted or further standard additions made by pipet "on-the-spot". The composite droplet is

then introduced by manually inserting the small bore plastic nebulizer tubing into the cone bottom. The conical shape guides the tube directly and reproducibly to the sample droplet thereby eliminating "waverling" of the hand or manual "bad aim" that might otherwise result in partial or interrupted aspiration of the drop. The drop "stands up" in easily accessible form in the cone owing to the shape of the vessel and its hydrophobic construction material. The tubing end is cut at approximately 10° - 20° to the normal to ensure that the resultant oval end will not seal against the side of the cone causing a sudden deleterious shift in the aspiration rate of the capillary nebulizer. The entire drop is readily aspirated due to its surface tension which makes it hold together as a "bead" even though the tubing does not seal against the side of the cone.

Both cones are rinsed between samples with a portion of distilled water, although it was found that droplet aspiration is normally so complete that six or more samples can frequently be run in succession without rinsing before a 1% cone contamination builds up. Rinse water is conveniently removed by aspiration so no Kim-"wiping" is necessary.

RESULTS AND DISCUSSION

Volume Response. Fig. 2 illustrates that micro-sampling is a reasonable and desirable technique for premix pneumatic nebulizers.

100 μL samples give approximately the same concentration sensitivity achieved with larger samples (e.g. 1 mL). The absolute sensitivity (weight basis) is however $\sim 10\times$ improved for a 100 μL sample. Samples smaller than 100 μL may be employed at constant absolute sensitivity and correspondingly reduced concentration sensitivity. Once a sample volume is chosen, conventional linear calibration curves are established at constant volume, or the method of standard additions may be employed. The data of Fig. 2 for the manual cone ("B") are in good agreement with the results of Sebastiani et. al (11) for mounted cones. This micro-sampling nebulizer approach was observed in these studies to produce a similar volume response for premixed flame emission and atomic fluorescence as well as atomic absorption spectrometry.

Precision. The precision of this method is generally independent of the mode of excitation employed and was measured in the present studies for atomic absorption (1 ppm Cd) to be $\sim 2\%$ RSD at a 100 μL sample volume. The micro-sampling precision of both cones (Fig. 1) in the range 50 - 200 μL was found to be essentially no different from that of steady state aspiration of larger (≥ 1 mL) samples. The precision worsens below 50 μL but does not exceed 10% RSD for samples as small as 10 μL for cone "A" and 5 μL for Cone "B".

The fact that this occurs for transient sampling suggests that it is totally unnecessary from a precision standpoint to aspirate large amounts of sample (≥ 1 mL) over extended periods of time for the purpose of averaging noise in the steady state signal. The improvement of cone "B" at volumes $< 50 \mu\text{L}$ is undoubtedly due to the fact that the sample pipetting is completed before any sample uptake occurs. The mounted cone (cone "A") however permits sample uptake to begin before pipetting is completed allowing interrupted uptake to produce undesirable multiple transients when the sample size drops below $10 \mu\text{L}$. Cone "B" therefore covers the microsampling range from $5\text{-}200 \mu\text{L}$ and cone "A" operates well from $10\text{-}200 \mu\text{L}$. The low cost and ease of construction should make it reasonable to obtain both cones as a set for an individual instrument. Samples larger than $200 \mu\text{L}$ are accommodated by either cone, but these and earlier (11,12, and 13) studies should make it clear that larger samples are generally not necessary on a peak height, analog readout basis.

New Micro-Calibration Technique (Cone "B" only); Dilution and Calibration. A Semiquantitative estimate on the first sample of a large group is performed using a $100 \mu\text{L}$ sample plus $10 \mu\text{L}$ water. (The $10 \mu\text{L}$ water is added to compensate a small percentage volume dilution by the subsequent $10 \mu\text{L}$ standard "add" in order to avoid uncorrected variation in the % matrix effect.)

If the resultant level is not suitable for the instrument scale factor, the factor is changed. If the level is altogether too high for atomic spectral quantitation, it is diluted "on-the-spot" by a known factor. This is accomplished without returning to the "wet bench" by using the same cone ("B") and set of micro-pipets used to introduce the sample into the spectrometer.

If the sample was approximately ten or one hundred times too high, 100 μ L or 1 mL respectively of water are quickly delivered to the empty (but rinsed) cone by Eppendorf pipet followed by a 10 μ L aliquot of sample. The composite is immediately aspirated to the last drop resulting in a rapid dilution by a precisely known factor. Proper solution mixing is induced on such a small scale by the pipetting action. Standard addition is then carried out following a quick semiquantitative estimation at the instrument (by electronic calculator if necessary) of the resultant concentration. This is accomplished by pipetting the same volume (equalling that (100 μ L or 1 mL) of the earlier used diluent water) of an appropriately selected aqueous standard into the empty (but rinsed) cone followed by 10 μ L of sample. The composite is again aspirated to the last drop and the signal increase noted at the recorder. Data are reduced later.

Direct Calibration. If the original undiluted 100 μ L sample trial (+ 10 μ L water) fell within the selected scale range, then that value is taken (instead of discarding it as an "estimate only"). To make a standard addition following semi-quantitative estimation of the initial reading, a 100 μ L sample is again pipetted into the cone followed by quick pipet addition of 10 μ L of an appropriately selected aqueous standard. The second pipetting action appears to mix the composite well. The composite is aspirated to the last drop and the signal increase noted on the recorder. Data are reduced later. The cone is rinsed once or twice in preparation for the next sample.

These studies have resulted in the ability to sit at the instrument with a large number of a variety of small volume digested complex samples of totally unknown concentrations that vary over many orders of magnitude and efficiently analyze them all by the method of additions without leaving the instrument for "wet bench" operations. Sample is conserved since the "add" is made directly following the initial estimation trial (before the calibration drifts) rather than discarding the signal used for estimation (as is normally done in favor of a later "rerun" when the analyst is ready to come back to the instrument following wet bench operations for the final calibration by standard addition).

An example of results using this procedure is shown in Fig.3. The claimed convenience of this procedure was borne out in the rapid determination of Pb, Cu, Zn, Fe, and Ca in digested complex samples including sea water, tomato sauce, blood, urine, orange juice, pineapple syrup, Grecian Formula 16, pickled beet juice, evaporated milk concentrate, air filters, orchard leaves, bovine liver, etc. The earlier report (11) of superior tolerance to complex materials (less nebulizer clogging) was also confirmed in the analysis of these high salt content digests.

Memory Effects. Two types of memory effects occasionally encountered in burner-nebulizer systems are illustrated in Fig.4. It should be apparent that any memory "spikes" such as those in part b. (Fig.4) will directly interfere with the transient micro-sampling technique. Since the steady state sampling method is not seriously affected by such memory, this will represent an interference disadvantage heretofore not mentioned in the literature for the micro-sampling approach.

The opposite is true of the second type of memory (Fig.4 parts c. and d.) which presents a more serious problem with steady state sampling, but is essentially eliminated (see Fig. 5) by the micro-sampling approach. The determination of silicon and iron in digested complex materials is frequently plagued by the second memory (not due to

reagent contamination) type and can be seen to be greatly improved by the small sample approach even if large amounts of sample are available. Fig. 5 also shows that the first memory type (spikes) generally only occurs when the sample acidity is suddenly dramatically raised (parts d., e., and f). The figure also shows that this memory becomes inconsequential (parts f. and g.) even at high acidity for micro-sampling if the sample acidity is maintained relatively constant thereafter.

Analysis Time. The micro-sampling cone was found to greatly increase the rate at which samples may be analyzed in comparison with steady state techniques. Eight replicate samples per minute were comfortably aspirated with the upper limit generally imposed by the pipetting speed rather than the system response. The high rate of speed (compared with steady state nebulization and electro-thermal atomization) that samples may be introduced by micro-sampling cone suggests that an automatic sample changer could be of great benefit with this technique. This would be useful mainly for large numbers of samples whose concentrations were all similar since most automatic sample changing schemes are not sophisticated enough to accommodate "on-the-spot" dilutions or standard additions.

CONCLUSIONS

The new micro-sampling cone for capillary pneumatic nebulization represents a technique of similar concentration sensitivity, improved

absolute sensitivity, similar precision, improved sample size requirements, improved analysis time, reduced memory problems and improved tolerance to complex materials in comparison with normal steady state aspiration approaches. Micro-sampling cones are therefore seen to be very desirable for premixed burner nebulizer systems used in flame emission and atomic fluorescence as well as atomic absorption spectrometry. It should be apparent that there is no longer as much need to turn to more troublesome thermal and electro-thermal atomization techniques for reasons of sample size alone since their range is also generally taken to be 5-100 μ l. The primary remaining reason for choosing thermal or electro-thermal atomization is therefore now that of sensitivity. There, however, appears to be no quantitative micro-sampling technique for atomic spectroscopy in existence that is collectively cheaper, more precise, or more convenient than the new micro-sampling nebulizer cone. The authors see no reason why all laboratories (even student labs) interested in sample analysis by flame spectroscopy should not own one each of the two cones for use with those trace levels for which nebulizer sensitivity is adequate.

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Figure 1. Micro-Sampling Cones for Premixed Capillary Nebulizer.

- a. mounted cone (Teflon) 10 μ L - 2mL capacity, 45° taper; b. manual cone (Teflon) 5 μ L - 2mL capacity, 45° taper.

Figure 2. Micro-Sampling Volume Response.

Figure 3. Example of "On-the-Spot" Micro-Dilution and Micro-Standard Addition for Orchard Leaf Digest (Cu).

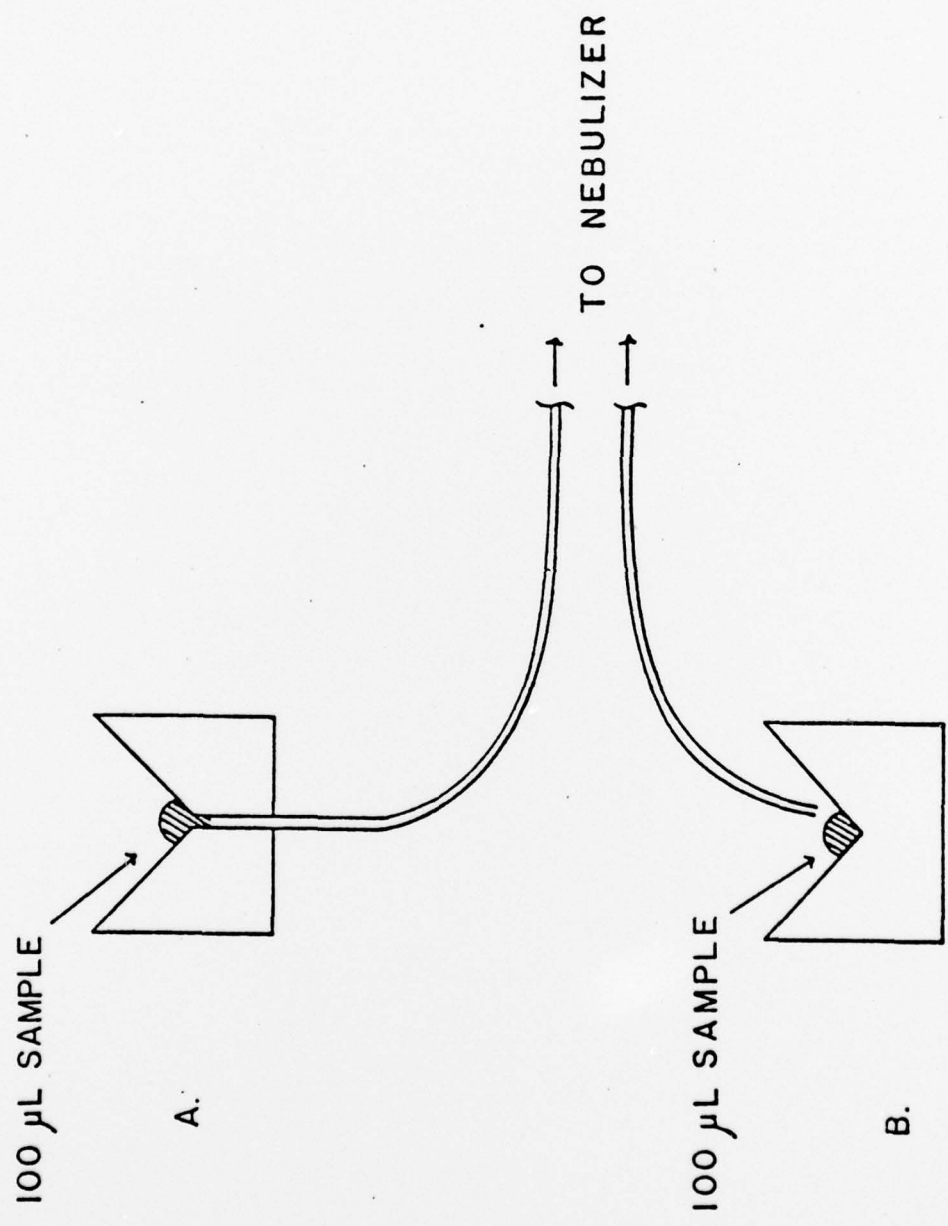
- a. blank; b. initial trial (too high); c. "on-the-spot" standard addition (+ 0.909 ppm duplicates); chart left running continuously.

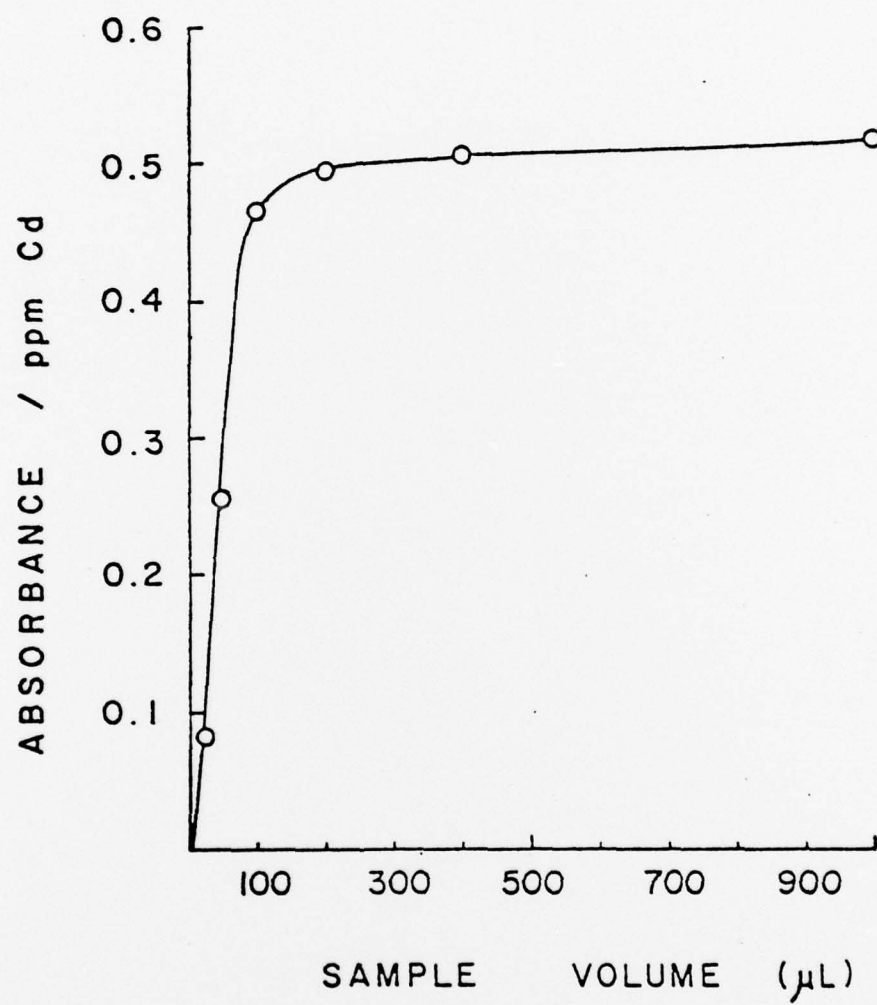
Figure 4. Type of Memory Encountered in Steady-State Sampling (Fe).

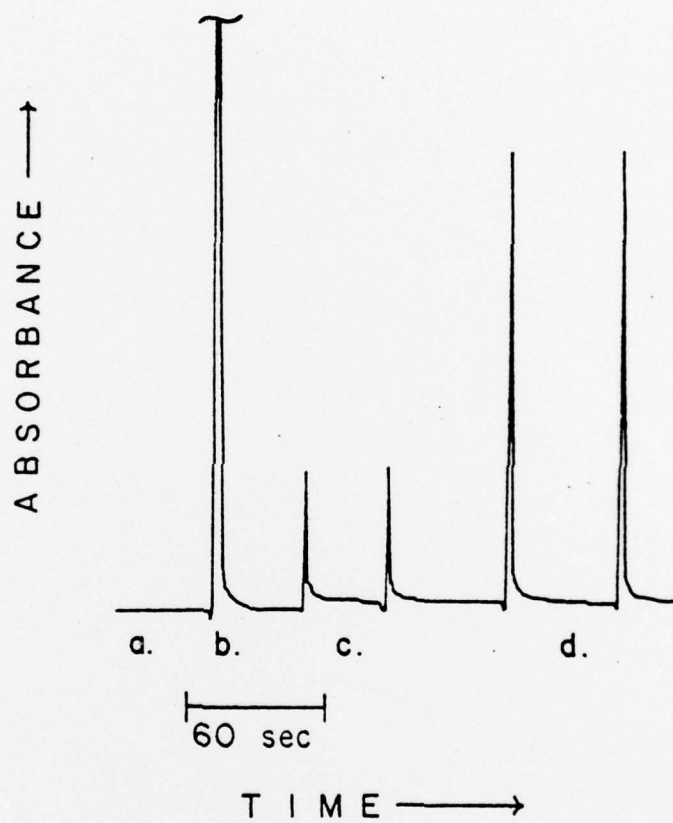
- a. normal steady state (0.2 ppm Fe in deionized water); b. "spike" memory accompanying sudden acidity increase; c. undetermined steady state (continually increasing signal) memory encountered with dilute acid (blank) for some elements; d. large continually increasing undetermined steady state memory encountered with conc. acid (blank) for some elements and burner systems.

Figure 5. Memory Effects.

- a. 0.2 ppm Fe in dil. mineral acid, steady state undetermined, analysis failed; b. 0.4 ppm Fe in dil. acid, steady state undetermined, analysis failed; c. acid blank, steady state undetermined; d. 0.2 ppm Fe in deionized water, steady state attained, analysis good; e. 0.4 ppm Fe in deionized water, steady state attained, analysis good; f. micro-sampling memory spikes (acid) accompanying sudden acidity increase; interference decays rapidly with trial number; g. greatly reduced acid blank encountered when using micro-sampling; h. 0.4 ppm Fe micro-sampling replicates in acid, analysis good; i. 0.2 ppm Fe micro-sampling replicates in acid, analysis good. Similar memory improvements were observed for micro-sampling analysis of petrified wood digest (HF) for silicon by atomic absorption spectrometry.



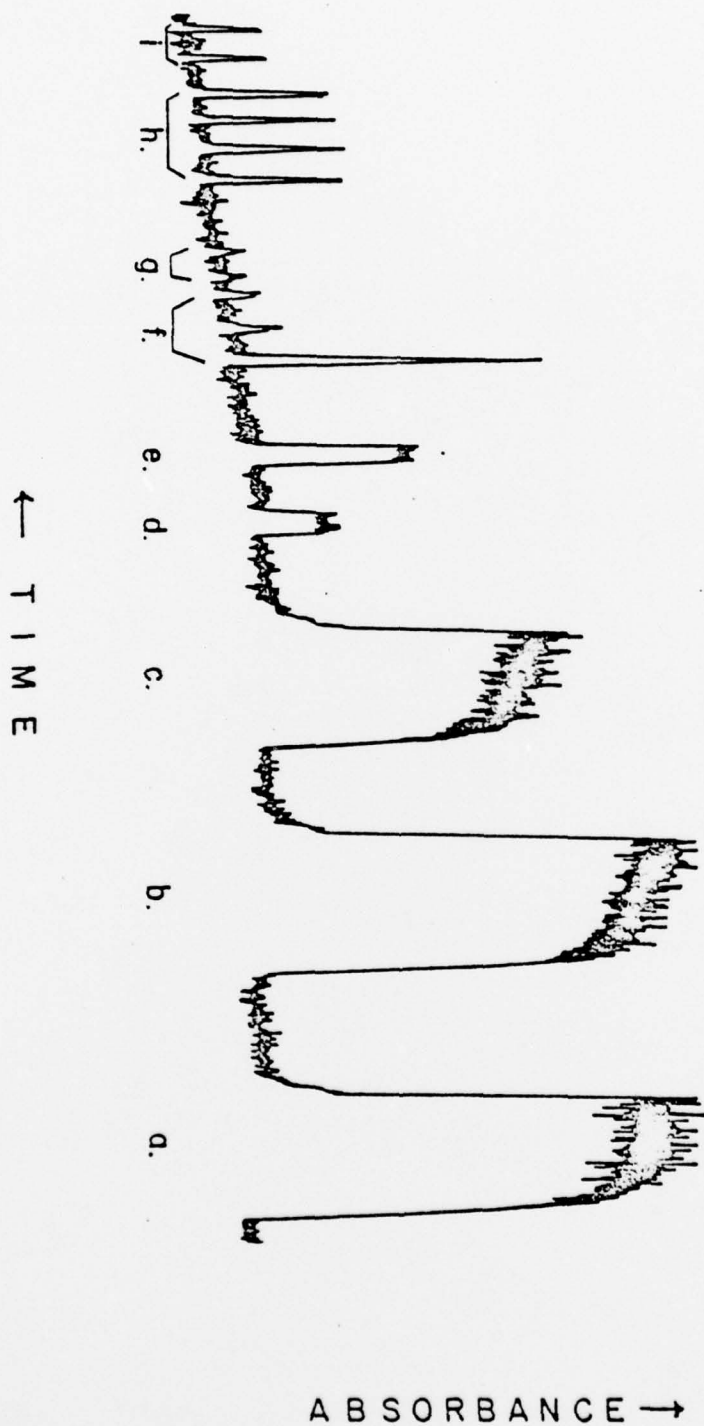






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